SHORT COMMUNICATION

A NEW LABORATORY MEDIUM FOR THE CULTIVATION OF *AGARICUS BISPORUS*

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SUMMARY

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Compost malt medium (CMM), a new, easily prepared laboratory medium for the cultivation of *Agaricus bisporus* is described. CMM is derived from an infusion of mushroom compost and provides *A. bisporus* with a growth medium which resembles its commercial substrate. The suitability of CMM as an artificial substrate for *A. bisporus* is demonstrated.

KEYWORDS: *Agaricus bisporus* - growth medium - microbial biomass - nutrition.

INTRODUCTION

Laboratory studies concerned with the biology of the cultivated mushroom, *Agaricus bisporus* (Lange) Imbach, are hampered by its poor growth on routinely employed media. Artificial substrates commonly used for the growth of *A. bisporus* include: malt extract agar (MEA; 2-4 % malt extract) (Hume & Hayes 1972, Wood 1976, San Antonio & Thomas 1972, Mathew 1961); potato dextrose agar (PDA) (Peerally 1979); complete yeast medium (CYM) (Elliott & Wood 1978); and commercially prepared malt extract (peptone) agar (MPA) (Masaphy *et al.* 1987), but the growth of the mycelium on these media is slow, and sectoring and strain degeneration are commonly seen (see the plates in Wood (1976)). Furthermore, phenomena observed on these media do not always reflect the situation *in vivo* (Rainey & Cole 1987). Media utilizing powdered compost, or compost extracts are sometimes used by spawn manufacturers and are reported to promote even growth (Fritsche 1978). These substrates however, are rarely used for laboratory studies.

Fungi are often grown on artificial media which bear little resemblance to their natural substrate (Tribe 1987). The media listed above, with the exception of compost agars, are all devoid of insoluble material and are rich in simple sugars, few of which are encountered by the mushroom on its 'natural' substrate. *A. bisporus* is usually grown on composted wheat straw/horse manure, a material which consists of a mixture of complex, mainly insoluble, plant and microbial residues. The insoluble fraction, which includes lignin, cellulose, hemicellulose, protein and microbial biomass, is preferentially used by the mushroom mycelium for growth (Fermor & Wood 1979).

The microbial biomass represents approximately 2 % of the compost dry weight (Sparling *et al.* 1982) and its importance as a source of nutri-

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ents for *A. bisporus* has recently been demonstrated (Fermor & Wood 1981, 1982, Grant *et al.* 1984, Fermor & Grant 1985). During composting, a dark brown, amorphous matrix of polysaccharide, microbial cells and debris accumulates on the straw surfaces (Eddy & Jacobs 1976, Atkey & Wood 1983). *A. bisporus* produces a range of extracellular enzymes, including bacterial cell wall degrading muramidases, which enable it to utilise this 'microbial matrix' as a concentrated source of nitrogen, minerals and carbon (Fermor & Grant 1985).

CMM was developed after consideration of the commercial substrate and growth requirements of the mushroom. The contribution of the insoluble fractions from the compost, especially the microbial biomass, to the nutrition of the mushroom were considered to be important.

MATERIALS AND METHODS

CMM was prepared as follows. A sample (5-10 kg) of fresh, evenly textured, commercially prepared, mushroom compost was oven dried (75°C for 2 days, or until no further weight loss was detected) and stored at room temperature. A 50 g sub-sample of the dried compost was added to 1000 ml of distilled water and the mixture left to infuse for 1 h. The temperature was raised to 100°C for 5 min and the mixture stirred vigorously to aid removal of the 'microbial matrix' from the straw surfaces. After a further 2 h infusion period the cooled mixture was filtered through four layers of muslin to recover approximately 800 ml of dark brown infusate. To this liquid 0.75 % malt extract (Oxoid L39) and 1.5 % Bacto agar (Difco B140) were added, and the pH was adjusted to pH 7.4 with 1 N NaOH. The medium was autoclaved (121°C, 15 min) and mixed well to ensure even distribution of the particulate matter, before pouring into Petri dishes.

The above formulation was adopted after conducting trials (results not shown) which examined the effect of a range of CMM components on the growth of *A. bisporus*. Included in these trials was an examination of the effect of different concentrations and sources of malt extract, the effect of different concentrations and sources of agar, and the influence of compost source, infusion methods and infusate concentrations on the growth of *A. bisporus*. The effect of adding nitrogen (organic and inorganic) and of incorporating a buffering system were also investigated.

The suitability of CMM as an artificial substrate for the growth of *A bisporus* was examined by comparing the radial extension rate of *A. bisporus* mycelium on CMM and on a range of frequently used laboratory media. The vigour, uniformity of growth and sectoring tendencies of mycelium were also assessed. The following media were used: 2 % MEA-Bacto (2 % Bacto malt extract, Difco B186; 1.5 % Bacto agar, Difco B140); 2 % MEA-Maltexo (2 % 'Maltexo' malt extract, Cerebos Gregg's Ltd., New Zealand; 1.5 % Bacto agar, Difco B140); PDA (Oxoid CM139); MPA (Oxoid CM59 - contains 0.5% peptone); the basal medium of Eggins & Pugh (1962) supplemented with 1 % glucose and solidified with 1.5 % Bacto agar (Difco B140); and CMM as described above. CMM from which the malt extract was omitted (compost extract agar (CEA)) was also used in the trial. All media were adjusted to pH 7.4 with 1 N NaOH and were sterilized by autoclaving. Disposable Petri dishes, 90 mm diam., were filled with 25 ml of medium (5 replicates per treatment) and centrally inoculated with a 5 mm agar plug removed from the margin of a 10 d culture of a commercial *A. bisporus* strain ('Horst U3', Somycel) growing on basal medium. Plates were incubated at 25°C, and colony diameter (the average of 2 perpendicular measurements) measured at 48 h intervals.

RESULTS AND DISCUSSION

The rate of linear extension of *A. bisporus* mycelium on the range of media listed above is shown in Fig. 1. CMM promoted rapid, sustained growth of vegetative mushroom mycelium, enabling complete colonisation of a 90 mm diam. Petri dish to occur within 2 wk. Radial growth of fungal colonies occurs at a constant linear rate (Cochrane 1958) provided there is an excess of all nutrients within the growth substrate, and an absence of inhibitory substances (Trinci 1984). On media other than CMM, the rate of linear extension of hyphae decreased once the Petri dish was approximately 50 % colonised (10 - 14 d). On

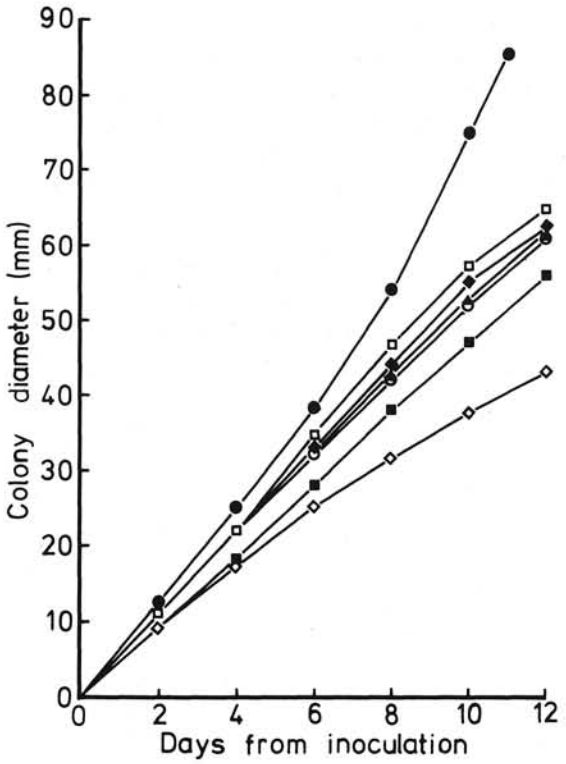

Figure 1. Colony growth of *A. bisporus* on CMM (●), MEA-Maltexo (□), PDA (◆), CEA (▲), MPA (○), basal medium (■) and MEA-Bacto (◇). Data are means of 5 replicates. S.E's are contained within the symbols.

CEA this decline in radial growth rate was not marked, but on 2 % MEA-Bacto, the rate of hyphal extension decreased to such an extent that 8-10 weeks was required for *A. bisporus* to fully colonise a 90 mm Petri dish. On 2 % MEA-Maltexo, complete colonisation of the Petri dish took three and a half weeks; this is comparable to the growth of *A. bisporus* on 2 % MEA (Boots Pure Drug Co., U.K.) reported by Wood (1976). These results demonstrate that substrates commonly used for the culture of *A. bisporus* are either unable to provide *A. bisporus* with the nutrients required to support a constant growth rate, or promote the build up of fungal metabolites which are inhibitory to the growth of the mycelium. The use of these media for routine culture of *A. bisporus* should be re-examined.

The vigour of the growth stimulated by CMM is shown in Fig. 2. Growth of *A. bisporus* was markedly affected by different media:- PDA and

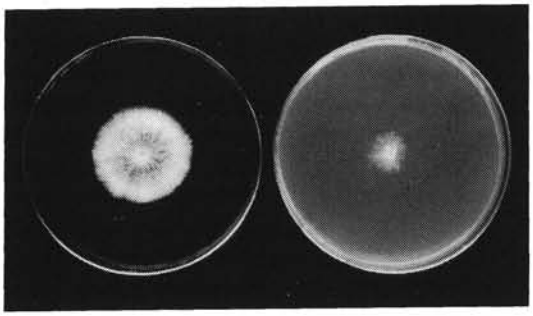

Figure 2. Growth of *A. bisporus* on CMM (left) and MEA-Bacto (right), 6 d after inoculation.

MPA both stimulated dense, uneven growth in which sectoring was apparent, 2 % MEA-Bacto promoted weak, uneven growth (Fig. 2) and the basal medium stimulated slow growth in which sectoring regions were common. On 2 % MEA-Maltexo and on CEA the growth of mushroom mycelium was even and few sectoring areas were seen. Growth on CEA, however, was sparse. Sectoring was rarely observed on CMM and growth was extremely vigorous.

The effect of different batches and sources of compost on the performance of CMM (determined by assessing the growth of *A. bisporus* on CMM) was negligible. CMM stimulated good growth in basidiomycetes other than *A. bisporus*, including: *A. bitorquis; A. campestris; Auricularia auricula; Coprinus bilanatus; Flammulina velutipes; Lentinus edodes; Pleurotus ostreatus; P. flabelatus* and *Volvariella volvaca*. CMM from which agar was omitted also served as a good growth medium for *A. bisporus*.

CMM is a useful laboratory medium for the growth of *A. bisporus* and consistently promotes rapid and vigorous mycelial development. The enhanced growth can be attributed to the medium's resemblence to the commercial substrate of *A. bisporus*. This property renders CMM invaluable for *in vitro* studies concerned with the biology of *A. bisporus*.

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